REVISED NATIONAL TUBERCULOSIS CONTROL PROGRAMME (RNTCP)

Module *for* Laboratory Technicians

September 1997

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Central TB Division, Directorate General of Health Services, Ministry of Health and Family Welfare, Nirman Bhavan, New Delhi 110 011

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Annexures

AIM OF MODULAR TRAINING

This module contains information on tuberculosis and sputum microscopy. The module includes exercises on activities and skills which the Laboratory Technician (LT) has to perform to implement the Revised National Tuberculosis Control Programme (RNTCP).

On successful completion of training, including hands-on training in a microscopy laboratory, the LT will be able to understand and perform all the job requirements related to the RNTCP.

WHAT IS TUBERCULOSIS?

Tuberculosis (TB) is an infectious disease caused by the bacterium, *Mycobacterium tuberculosis*. Tubercle bacilli mainly affect the lungs, causing lung tuberculosis (pulmonary tuberculosis). However, in some cases, other parts of the body may also be affected, leading to extra-pulmonary tuberculosis.

HOW DOES TUBERCULOSIS SPREAD?

TB germs usually spread through the air. When a patient with pulmonary tuberculosis coughs, sneezes, or talks he throws TB germs into the air in the form of tiny droplets. These tiny droplets when inhaled by another person may spread TB. When patients with tuberculosis begin taking effective treatment, they stop spreading the germs within a few weeks. But unless they take the treatment regularly and complete it, they are likely to develop more dangerous forms of tuberculosis, known as drug-resistant tuberculosis, which they can then spread to others.

MAGNITUDE OF TUBERCULOSIS IN INDIA

Some important facts you should know:

1. It is estimated that about 140 lakh people are suffering from tuberculosis in our country, of whom about 35 lakh are sputum-positive and highly infectious.

2. About 22 lakh cases of tuberculosis are added every year out of which about 10 lakh are sputum-positive.

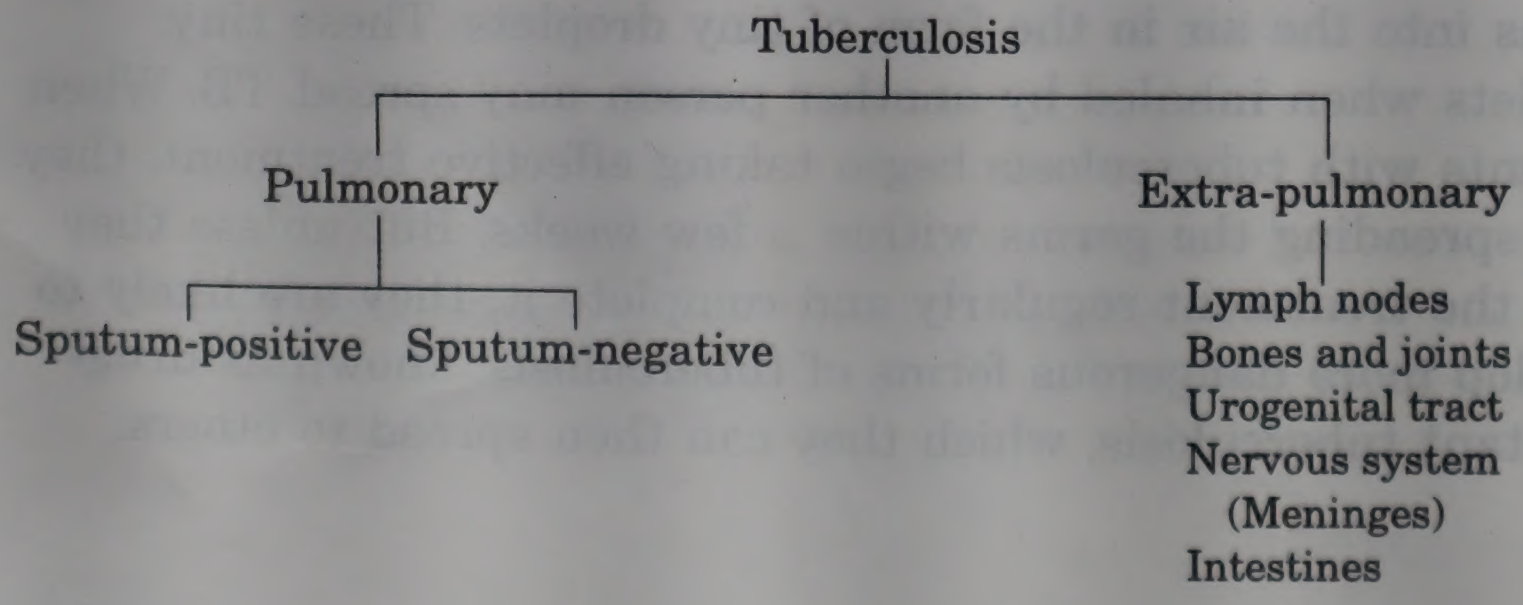
3. About 5 lakh people in India die due to tuberculosis every year, i.e. one patient with tuberculosis dies every minute; more than 1000 people die every day.

4. Unless treated effectively, one sputum-positive patient can infect 10–15 individuals in one year.

It is estimated that in a year, 135 cases of tuberculosis per 100,000 population will be diagnosed and treated under the RNTCP.

CLASSIFICATION OF TUBERCULOSIS

Please try to understand the following chart:



PULMONARY TUBERCULOSIS

Sputum smear-positive

A patient with at least 2 initial sputum smear examinations positive for Acid-Fast Bacilli (AFB),

Or: TB in a patient with one sputum examination positive for AFB and radiographic abnormalities consistent with active pulmonary TB as determined by the treating Medical Officer,

Or: TB in a patient with one sputum specimen positive for AFB and culture positive for *M. tuberculosis.*

Sputum smear-negative

Tuberculosis in a patient with symptoms suggestive of TB with at least 3 sputum examinations negative for AFB, and having radiographic abnormalities consistent with active pulmonary TB as determined by the MO, followed by a decision to treat the patient with a full course of anti-tuberculosis therapy,

Or: Diagnosis based on positive culture but at least 3 sputum examinations negative for AFB.

A sputum smear-positive patient with AFB detected by microscopy is much more infectious than a patient who does not have AFB detected by microscopy. Nevertheless, even patients with sputum smears negative for AFB can have pulmonary tuberculosis.

EXTRA-PULMONARY TUBERCULOSIS

A patient with history and/or clinical findings consistent with active tuberculosis of any part of the body other than the lungs is a case of extra-pulmonary tuberculosis. Diagnosis and treatment of such a case will be done by the Medical Officer.

TB can affect any part of the body.

WHEN SHOULD TUBERCULOSIS BE SUSPECTED?

Pulmonary tuberculosis

Symptoms suggestive of pulmonary tuberculosis are:

1. Cough with expectoration for 3 weeks or more
2. Rise of temperature in the evening
3. Chest pain
4. Weight loss
5. Loss of appetite
6. Haemoptysis (coughing up of blood in sputum)

The most common symptom of pulmonary tuberculosis is persistent cough for 3 weeks or more (usually with expectoration which is sometimes blood-stained) with or without associated fever and chest pain. Every patient with cough for 3 weeks or more should have 3 sputum samples examined for AFB.

Extra-pulmonary tuberculosis

In persons with extra-pulmonary tuberculosis, the symptoms depend on the organ involved.

- Lymph Node Tuberculosis—Swelling in the neck with or without discharge.
- Tuberculous Meningitis—Headache, fever, drowsiness, confusion, neck rigidity.
- Spinal Tuberculosis—Back pain, fever and in some cases swelling of the backbone.

HOW SHOULD TUBERCULOSIS BE DIAGNOSED?

Detection of AFB in the sputum is the only reliable method for confirming pulmonary tuberculosis. Therefore, the LT plays a pivotal role in the diagnosis of patients and thus in the success of the programme.

Cases of pulmonary tuberculosis are further divided into sputum smear-positive and sputum smear-negative cases. When pulmonary tuberculosis is suspected, sputum must be examined to confirm the diagnosis. **Three sputum samples (spot—morning—spot)** should be collected, preferably within two days, and examined by microscopy.

A person with at least two positive sputum smear samples is a confirmed case of sputum smear-positive pulmonary tuberculosis.

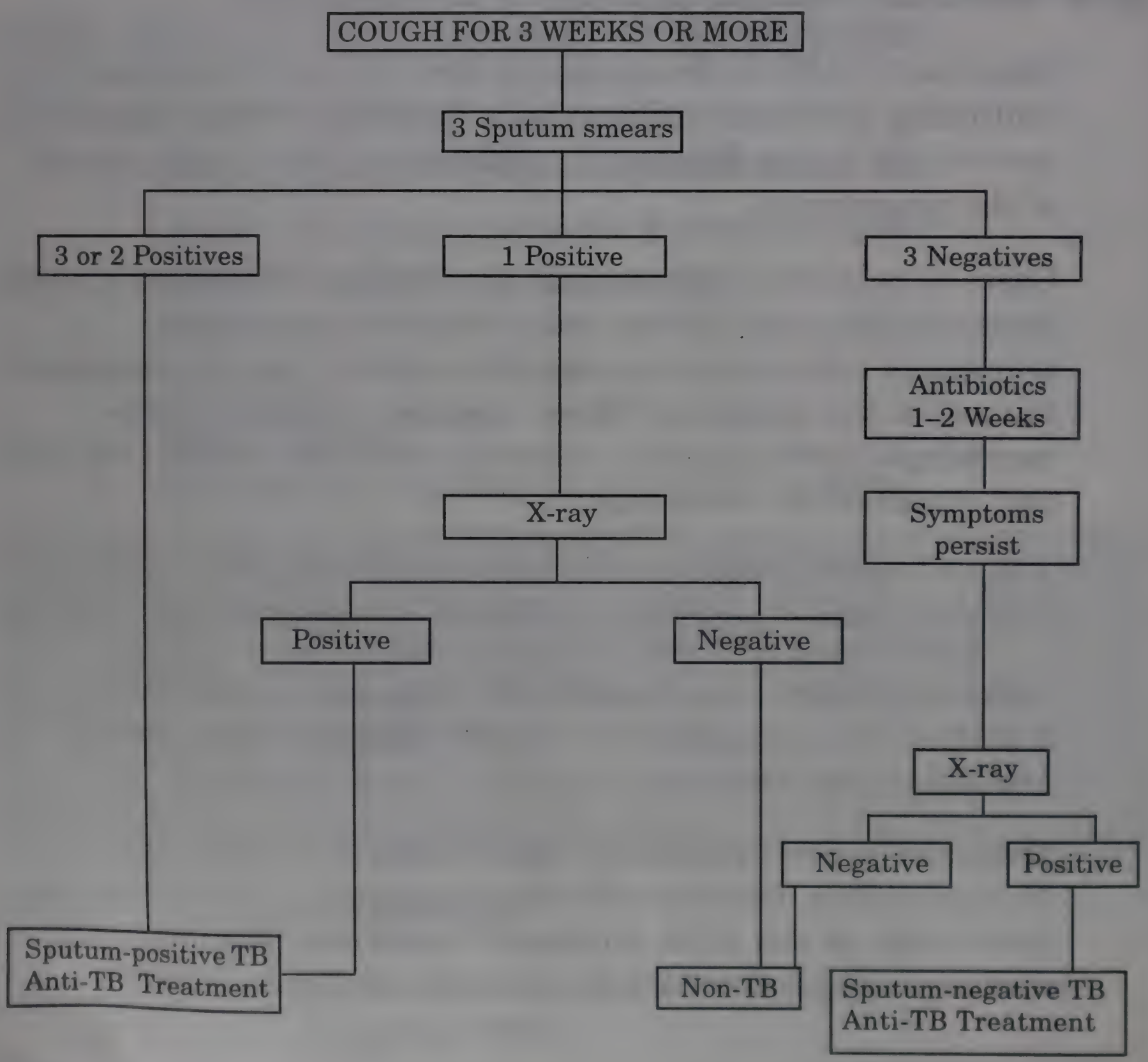
Patients who are smear-negative or have only one smear out of 3 positive for AFB are referred to the Medical Officer for clinical evaluation and X-ray.

Diagnosis of extra-pulmonary tuberculosis is made by the Medical Officer. Patients with extra-pulmonary tuberculosis who have cough or any other pulmonary symptoms should have 3 sputum samples examined to determine if they also have

pulmonary tuberculosis. A patient may have both pulmonary and extra-pulmonary tuberculosis.

The approach to diagnosing patients with possible tuberculosis is summarized in the diagram below:

Diagnosis of TB in Chest Symptomatics



Patients who have cough for three weeks or more or other symptoms of tuberculosis must be evaluated for tuberculosis by sputum microscopy. It is estimated that 2–3% of adult outpatients are chest symptomatics. The LT has to ensure that testing of sputum is accurate and results of sputum microscopy are passed on to the treating physician at the earliest, preferably within a day.

NATIONAL TUBERCULOSIS PROGRAMME

The National Tuberculosis Programme (NTP) in India was implemented in 1962 by establishing District Tuberculosis Centres (DTCs), TB Clinics and TB Hospitals. Since its inception, the programme was integrated with the general health services and service delivery was through the primary health care infrastructure.

To strengthen and improve tuberculosis control activities, the Government of India has launched the Revised National Tuberculosis Control Programme (RNTCP) in a phased manner. Beginning in 1997, the programme will be expanded to cover nearly one third of the country over three years.

REVISED NATIONAL TUBERCULOSIS CONTROL PROGRAMME

	Objectives	Strategies to be adopted
1	To achieve at least 85% cure rate among the new sputum smear-positive TB cases registered.	• All cases diagnosed must be registered. All registered cases must be treated till they are cured, with priority for cure given to sputum smear-positive cases. • Directly Observed Treatment, Short-Course Chemotherapy (DOTS), with observation of treatment done close to the patient's home. • Maintain regular drug supply. • Ensure stipulated smear examinations at specified intervals to monitor progress and cure of the patient.
2	To detect at least 70% of the estimated new sputum smear-positive cases after achieving objective No. 1.	• Diagnosis by sputum microscopy among patients attending health services. • Ensure that all persons attending health facilities with cough or other symptoms of tuberculosis have 3 sputum smears examined. • Ensure high quality sputum microscopy and prompt reporting of results.

WHAT IS DOTS, CURE AND CURE RATE?

DOTS

As the name implies, Directly Observed Treatment, Short-Course Chemotherapy (DOTS) means that the patient swallows short course anti-TB drugs in the presence of a health worker or other trained individual. There are two phases in the treatment of tuberculosis: the **intensive phase**, which is for 2 or 3 months, and the **continuation phase**, which is for 4 or 5 months. The length of treatment depends on the category of treatment the patient is taking. Under the programme, these drugs are swallowed thrice-a-week on alternate days during the intensive phase. Thereafter, the sputum is examined and if found negative, the patient is issued anti-TB drugs once a week in a weekly calendered multiblister combipack to be taken on alternate days (thrice-a-week). The first dose from the weekly pack is to be taken in the presence of a health worker. Drugs for the rest of the week are taken by the patient on alternate days. The intake of drugs by the patient at his house is monitored by checking the empty blisterpacks during the time of collection of drugs for the next week. Sputum examination must be done after two months in the continuation phase, and at completion of treatment.

Advantages of DOTS

- **Places responsibility for patient cure on the health worker, not on the patient**
- **A service to patients**
- **Prevents drug-resistant TB**
- **Reduces risk to the community by preventing spread of TB**
- **Cost-effective**
- **The only method which ensures cure**

Cure

A patient who was initially sputum smear-positive, and who has completed treatment and has negative sputum smears on at least two occasions, including one at completion of treatment, is declared as cured. If the sputum is not examined during and at the end of treatment, then the patient is said to have **completed** the treatment. To be declared **cured**, sputum examinations are essential.

Cure Rate

The cure rate is the proportion of initially sputum smear-positive patients who are declared as cured based on negative sputum smear results on at least two occasions, including one at the end of treatment. The cure rate of new sputum smear-positive patients is the most important indicator of the success of the programme. The goal of the RNTCP is to ensure that this proportion is at least 85%.

Treatment for tuberculosis is highly effective, if it is taken regularly for the prescribed period. The Medical Officer will classify the patient into one of three treatment categories—Category I, Category II or Category III. The category in which the patient is classified determines the drugs to be given and the schedule for sputum examinations.

Detailed job responsibilities of the LT are given in the Laboratory Manual and reproduced in Annexure VII.

EXERCISE 1

Tick the ONE most appropriate answer.

1. Tuberculosis is transmitted by

   (a) Blood transfusion _____
   (b) Faecal infection _____
   (c) Droplet infection _____
   (d) Oral infection _____

2. The most infectious form of tuberculosis in adults is

   (a) Extra-pulmonary TB _____
   (b) Smear-positive pulmonary TB _____
   (c) Smear-negative pulmonary TB _____
   (d) Miliary TB _____

3. The commonest form of tuberculosis is

   (a) Extra-pulmonary TB _____
   (b) Bone and joint TB _____
   (c) Renal TB _____
   (d) Pulmonary TB _____

4. When do you suspect pulmonary tuberculosis? Mention the four commonest symptoms.

   (i) _____
   (ii) _____
   (iii) _____
   (iv) _____

5. How do you classify tuberculosis?

(i) _____

(ii) _____

6. Which is the surest way to diagnose pulmonary tuberculosis in an adult?

(a) Sputum smear examination _____
(b) X-ray _____
(c) ELISA test _____
(d) Tuberculin skin test _____
(e) ESR _____

7. When do you label a patient as a case of smear-positive pulmonary tuberculosis?

_____

8. TB can affect any part of the body.

❑ True ❑ False

9. Patients with three sputum smears negative for AFB cannot have pulmonary tuberculosis.

❑ True ❑ False

IMPORTANT POINTS TO REMEMBER

- **All patients with symptoms of pulmonary tuberculosis should have three sputum examinations done for AFB. By identifying patients with tuberculosis promptly and correctly, severe illness, death and spread of the disease can be prevented.**
- **Patients with negative sputum smears can have pulmonary tuberculosis. However, these patients are less infectious than patients with positive sputum smears.**
- **The most common symptom of pulmonary tuberculosis is persistent cough for 3 weeks or more.**
- **Other symptoms of pulmonary tuberculosis include weight loss, fever, night sweats, chest pain, loss of appetite and coughing up blood in the sputum.**
- **With effective, regular and complete treatment, TB can be cured. Documenting cure requires sputum examination by microscopy. Cure rate is the most important indicator of the success of the programme. The national goal is to achieve a cure rate of at least 85%.**

COLLECTING SPUTUM, PREPARING AND STAINING SLIDES, EXAMINING SLIDES, AND RECORDING, REPORTING AND VERIFYING RESULTS

The process of collecting sputum, preparing and staining slides, examining slides, and recording, reporting and verifying results can be divided into six stages.

Stages of sputum smear examination are as follows:

1. Collect the sputum
2. Prepare the slide for examination
3. Examine the slide under the microscope
4. Record the results
5. Report the results
6. Verify the results.

STAGE 1: COLLECT THE SPUTUM

Receive patient and Laboratory Form. Make sure the form is complete.

The patient should have been referred by a Medical Officer. If patients approach you directly without visiting the Medical Officer, you should ensure that they are seen by a Medical Officer.

You must make sure that the Laboratory Form is complete, including the patient's address and reason for examination. Confirm the address of the patient again so that the patient is not lost if follow up is required. If the sputum is for follow-up examination, the patient's TB Number should have been written on the form.

Reason for Examination. If the patient has come to the health facility for the first time, the sputum is examined for diagnosis. In this case, 3 sputum samples are examined (SPOT—MORNING—SPOT). After a patient is diagnosed as a case of tuberculosis, treatment is started. For follow-up examinations, two samples are obtained (MORNING—SPOT). The schedule for sputum examinations is summarized in the table below.

The results of follow-up sputum examinations are important. The treatment a patient receives depends on these results. If the first follow-up sputum examination is positive (that is, after the second month of starting treatment for patients receiving Category I treatment, or after the third month of starting treatment for patients receiving Category II treatment), then the treatment is extended for one more month and the sputum is examined again after that.

Schedule of Sputum Examinations

Category of treatment	Schedule of follow-up sputum examinations
Smear-positive Category I	At the end of 2, 4 and 6 months of treatment
Smear-positive Category I (If sputum-positive at the end of Month 2)	At the end of 2, 3, 5 and 7 months of treatment
Smear-positive Category II	At the end of 3, 5 and 8 months of treatment
Smear-positive Category II (If sputum-positive at the end of Month 3)	At the end of 3, 4, 6 and 9 months of treatment
Smear-negative Category I or Category III	At the end of 2 and 6 months of treatment

Specimen Identification Number. This number is not given to patients whose sputum is collected at the microscopy centre. This number is given only by health workers and others who

are collecting sputum specimens and transporting the containers to your centre for microscopic examination. You will give all patients a Laboratory Serial Number, whether or not there is a Specimen Identification Number.

Patient's TB Number. All patients diagnosed as suffering from tuberculosis are entered in the TB Register maintained by the Tuberculosis Unit. The TB Number is very important. If a patient's sputum is being examined for follow-up, the TB Number should have been written in the space provided. The TB Number should also appear on the patient's Identity Card. If the patient is carrying this card, you can enter the number from this card if it has been omitted from the Laboratory Form.

Demonstrate to the patient how to open and close the sputum container and how to bring up sputum

Give the patient the sputum container with the Laboratory Serial Number written on its side. Show the patient how to open and close the container and explain the importance of not rubbing off the number you have written on the side of the container.

Explain to the patient that sputum examination is the only sure way to confirm the diagnosis of pulmonary tuberculosis. If it is convenient, you may show AFB-positive slides under the microscope to the patient.

Demonstrate to the patient by actions how he should bring up sputum. Most people do not understand the difference between saliva and sputum. Explain to the patient the characteristics of sputum: that it is thick and mucoid, as compared to saliva which is thin and watery.

Please review your Laboratory Manual for detailed instructions on collecting sputum samples.

Write information on the Laboratory Form and on the Sputum Containers

Laboratory Serial Number. A new Laboratory Serial Number is assigned to each of the chest symptomatics whose sputum is examined. The Laboratory Serial Number begins with 1 on 1 January each year and increases by one with each patient until 31 December of the same year. Each set of samples (3 for diagnosis, 2 for each follow-up examination) is given *one* Laboratory Serial Number. In other words, the serial number is given to a *set* of slides and not to individual slides. Enter the *Laboratory Serial No.* on the sputum container and the Laboratory Form.

It is important to label sputum containers properly. Sputum containers should always be labelled on the side, and never on the lid, as the lid from one container may be placed on another container resulting in specimens being labelled incorrectly. If the labelling is incorrect, a patient who should have been treated may not get treatment while a patient who does not have TB may be put on treatment unnecessarily. Label clearly with a marker that will not be easily erased.

Check the sample to see if it is sputum or saliva only

You must make sure that the sputum sample is of good quality for microscopic examination. Please review your Laboratory Manual for information on how to determine whether samples are of good quality.

If the sputum sample is good, the chances of finding AFB are greater. If the sputum sample is only saliva, microscopic examination may be falsely negative for AFB. Poor quality sputum samples will result in patients receiving incorrect treatment or no treatment at all. In this case, patients may become seriously ill or die, and also spread tuberculosis to their family and community. For this reason, it is important that you visually examine every sputum sample and record its appearance on the Laboratory Form.

If the sample is poor, ask the patient to cough again until a good sample is obtained. It may take several minutes for the patient to bring out a good specimen.

If you have explained carefully and demonstrated to the patient how to bring out sputum, samples will be of good quality and you will not need to request additional sputum for examination.

Write the visual appearance of the sputum sample on the Laboratory Form

Write the visual appearance of the sputum sample on the Laboratory Form in the space provided for it. You must make sure that the Laboratory Serial Number on the Laboratory Form is the same as the Laboratory Serial Number on the sputum container.

If the patient has provided a sample which is saliva, explain again the importance of a good sputum sample brought out from deep within the lungs. Demonstrate how to bring up sputum, and ask the patient to provide another sample. A patient whose sputum is to be examined for follow-up may only be able to produce saliva, despite good efforts to produce sputum. These efforts should include having the patient take a series of deep breaths. If this is not successful, try patting the patient gently on the back to help him bring out sputum. If this is not successful, ask the patient to drink something warm and then try to bring out sputum again. If, despite these efforts, the patient is still only able to produce saliva, then the saliva should be examined and the results recorded.

STAGE 2: PREPARE THE SLIDE FOR EXAMINATION

The next steps regarding preparing, staining, examining and reporting a sputum smear are summarized below.

Label the slide with the Laboratory Serial Number

When you are ready to prepare the smear, label the slide with the Laboratory Serial Number on the left side. This must be written only with a diamond marker or grease pencil.

Remember that a new Laboratory Serial Number is assigned to each chest symptomatic whose sputum is examined. The Laboratory Serial Number begins with 1 on 1 January each year and increases by one with each patient until 31 December of the same year. Each set of samples (3 for diagnosis, 2 for each follow-up examination) is given the same Laboratory Serial Number.

Be careful not to leave fingerprints on the slide. Fingerprints can interfere with staining and make accurate examination difficult under the microscope. Only new slides should be used for AFB microscopy, because scratches on old slides can look like AFB, giving a false-positive result.

Spread sputum on the slide using a broomstick and stain the slide

Spreading the sputum correctly on the slide is essential for good staining and accurate microscopic examination.

Explanation of Steps in the Preparation and Staining of Slides

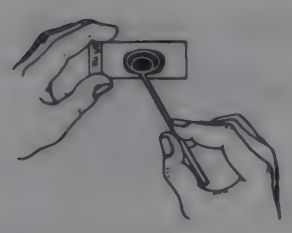
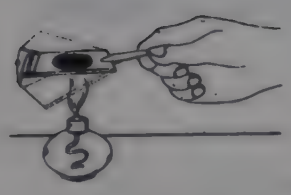
Illustration	Steps of the staining procedure	Reasons for/Comments on each step
	Spread sputum on the slide using a broomstick	• If samples are spread too thickly or too thinly, staining and microscopic examination will not be accurate. • A different broomstick is used for each smear so that one patient's sputum is not mixed with another patient's sputum.
	Allow the slide to air dry for 15–30 minutes	• Heating the slide while it is wet could result in bubbling of TB bacilli into the air.
	Fix the slide by passing it over a flame 3–5 times for 3–4 seconds each time	• Fixation makes the sputum stick to the glass slide. • Fixation preserves the shape of the bacilli. • Heating for too long a period could change the shape of the bacilli and also cause the slide to break. • Heating for too short a period can result in a false-negative result because the TB bacilli will not be well preserved on the slide.

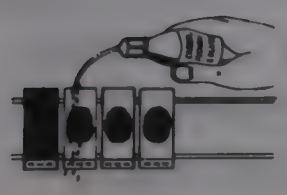
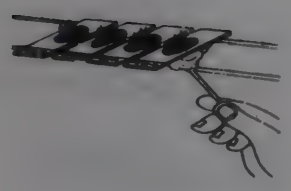
Illustration	Steps of the staining procedure	Reasons for/Comments on each step
	Pour filtered carbol fuchsin to cover the entire slide	• Carbol fuchsin stains the TB bacilli red. • The carbol fuchsin solution must be filtered before use. If it is not filtered, small particles (sediments) get poured onto the slide and can appear red like TB bacilli under the microscope.
	Gently heat the slide with carbol fuchsin on it until vapour rises. DO NOT BOIL.	• Carbol fuchsin solution must not be allowed to boil or to dry on the slide, otherwise it will form small particles resulting in a false-positive reading. These particles may look like TB bacilli. • When the slide is heated to 80–90°C, the carbol fuchsin on the slide penetrates the wall of the TB bacilli to stain the bacilli red. • Allowing the carbol fuchsin to boil will change the shape of the TB bacilli and may result in a false-negative reading.
	Leave carbol fuchsin on the slide for 5 minutes	• The wall of the TB bacillus is thick and waxy. It is essential to give carbol fuchsin sufficient time to penetrate the wall so that it can stain the bacilli.

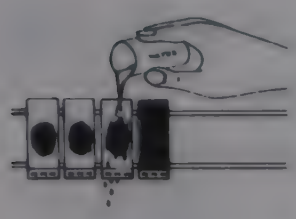
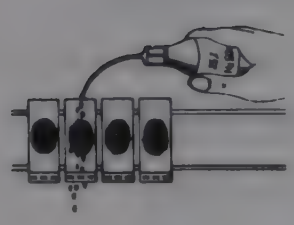
Illustration	Steps of the staining procedure	Reasons for/Comments on each step
	Rinse GENTLY with tap water until all free carbol fuchsin stain is washed away	• If water is poured too vigorously, the smear itself will be washed off the slide.
	Tilt the slide to drain off excess water	• If water is not drained off, it will dilute the next stain/reagent that is poured, reducing the effectiveness of the next step.
	Pour 25% sulphuric acid onto the slide	• Sulphuric acid removes carbol fuchsin stain from all of the contents of sputum except the TB bacilli. For this reason, TB bacilli are known as AFB, or Acid-Fast Bacilli, because the red colour of the AFB from the carbol fuchsin remains after they are decolourized with sulphuric acid.
	Let the slide stand for 2–4 minutes	• Allowing the slide to stand gives sulphuric acid time to wash out stain from everything except the TB bacilli. • If insufficient time is given, bacteria and sputum contents other than TB bacilli may retain their stain, giving a false-positive result.

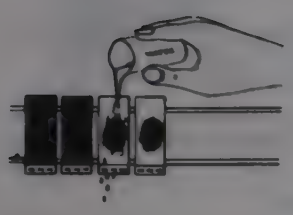
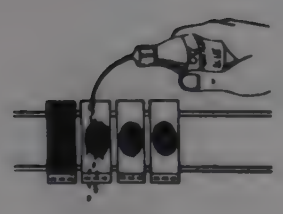
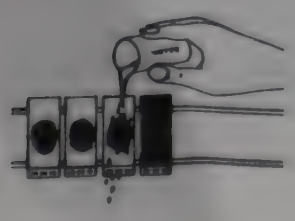
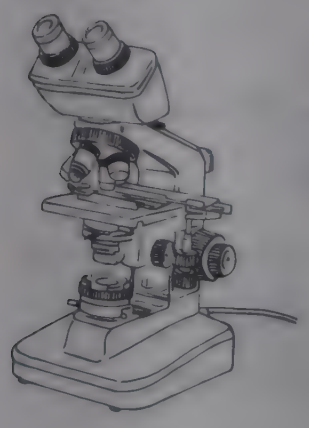
Illustration	Steps of the staining procedure	Reasons for/Comments on each step
	Rinse GENTLY with tap water	• Rinsing too strongly can wash the smear itself off the slide. • Sulphuric acid burns the skin. Do not let it splash. • If the slide is still stained red, you may apply sulphuric acid for a second time, letting the slide stand for 1–3 minutes this time. • It is helpful to use sulphuric acid to clean the bottom of the slide, on the opposite side of the smear. This makes it easier to examine the slide under the microscope.
	Pour 0.1% methylene blue onto the slide	• Methylene blue is the counterstain. It colours everything on the smear blue except the AFB. • The contrast between the AFB which are stained red by the carbol fuchsin and the rest of the smear stained blue by the methylene blue makes it easier to view the TB bacilli.
	Leave methylene blue on the slide for 30 seconds	• It takes about 30 seconds for methylene blue to stain the material on the slide.
	Rinse GENTLY with tap water	• Always rinse gently so that the smear is not washed off the slide.

Illustration	Steps of the staining procedure	Reasons for/Comments on each step
	Allow the slide to dry and then examine it under the microscope	• Examining a slide when it is still wet may damage the microscope. • Examining a wet slide will also make it difficult to focus the microscope and read the slide correctly. • Do not dry the slides by blotting.

Keep sputum cups and other materials in a safe place until they are discarded

Place the sputum containers, broomsticks, and other contaminated materials in a container with a foot-operated lid. The container should be filled with 5% phenol or freshly prepared 5% hypochlorite (bleach).

Do not dispose of sputum containers until you have examined the slides. In this way, if a repeat smear needs to be prepared from the same specimen, you can do so. However, as soon as all slides are examined, you must dispose of all contaminated materials, including sputum containers.

STAGE 3: EXAMINE THE SLIDE UNDER THE MICROSCOPE

Step-by-step examination of the slide, and reasons for/comments on these steps, are summarized in the table below.

Step	Reasons for/Comments on each step
Put one drop of immersion oil on the left edge of the stained smear	• Immersion oil is necessary for observations under the x100 lens. The oil will bridge the gap between the slide and the lens. • Never let the immersion oil applicator touch the slide. Doing so may contaminate the applicator. If the applicator touches the slide, you may spread AFB from one slide to the next resulting in false-positive results.
Bring the slide into focus with the x40, then the x100 lens	• The x40 lens allows you to find a suitable area of the slide to examine. Use the coarse focusing knob for this purpose. • After finding a suitable area, focus the x100 lens with the fine focusing knob. Do not use the coarse focusing knob for final adjustment, as it may break the slide and damage the microscope. • Never let the lens touch the slide. Doing this will damage the lens and may break the slide. In addition, the lens may pick up pieces of sputum and transfer them onto the next slide examined, giving a false-positive result.
Systematically examine at least 100 fields	• Even the most experienced microscopist needs to examine each slide for at least five full minutes. If you examine a slide for too short a period or not carefully enough, you may miss AFB which are present and report the result as negative when it is actually positive. Examine every slide as if it were from one of your family members.

Step	Reasons for/Comments on each step
	• If 1–9 bacilli are found in 100 oil immersion fields, examine another 100 oil immersion fields. • The appearance of AFB is shown in Annexure III of your Laboratory Manual.
Read results as: negative, scanty, or positive (1+, 2+ or 3+)	• See the Table below for grading and number of fields to be examined. • Grading of sputum smear results is an indicator of the load of infection and also provides epidemiological information.

Grading of Slides in AFB Microscopy

Examination	Result	Grading	Number of fields to be examined
More than 10 AFB per oil immersion field	Positive	3+	20
1–9 AFB per oil immersion field	Positive	2+	50
10–99 AFB per 100 oil immersion fields	Positive	1+	100
1–9 AFB per 100 oil immersion fields	Scanty	Record exact number seen	200
No AFB in 100 oil immersion fields	Negative	0	100

STAGE 4: RECORD THE RESULTS

The table below summarizes the steps in reporting results, and the reasons for each of these steps.

Step	Reasons for/Comments on each step
Verify the Laboratory Serial Number on the slide and record the result on the Laboratory Form	• Recording results properly is as important as staining and examining a slide correctly. Carelessness can harm patients as well as the programme itself. • Always write the date of the report and sign your name.
Wipe the x100 lens with lens paper	• The x100 lens is a delicate piece of equipment. • Oil will gradually damage the lens unless it is promptly and carefully wiped off after each session of use. • If you take good care of the microscope, it will last for many years. • Use a small amount of xylene to clean oil off the lens after each session of use. • Never use spirit to clean the lens, as this may damage it by dissolving the glue.
Write results from the Laboratory Form in the Laboratory Register	• For new patients, make sure the address is recorded correctly in the Laboratory Register. • You must enter the TB Number in the space provided for all patients whose Reason for Examination is Follow-up. This number should have been recorded on the Laboratory Form, and allows for cross-checking between your Laboratory Register and the Tuberculosis Register. • Every specimen MUST be entered in the Laboratory Register, regardless of where the patient resides or is treated. • All positive results should be written in the Laboratory Register with a **red pen**. This allows one to find all positive results quickly.

STAGE 5: REPORT THE RESULTS

Send the completed Laboratory Form back to the treating physician for information and necessary action. It is important to report these results within one day. The patient's treatment depends on these results, and any delay reduces the value of all the work you have done in examining a slide correctly.

If the patient has been referred from, and will begin treatment at the health unit where the microscopy centre is located, give the results to the treating physician. If the patient was referred from another health unit, make a duplicate copy of the Laboratory Form for the patient and send the original to the treating physician at the referring health centre.

Never give results only to the patient. If the patient fails to bring the results to the Medical Officer or Treatment Centre, he may not receive treatment.

STAGE 6: VERIFY THE RESULTS—PRESERVE SLIDES FOR REVIEW BY THE SUPERVISOR

Do not discard any slide until it has been reviewed by your supervisor. Wash the slide gently with xylene and place in the box containing examined slides. Xylene will not damage the slide or the stain, but will allow you to store it neatly and cleanly. If you do not wash the slides with xylene, they may stick together and it will not be possible for you or your supervisor to review them at a later date. Do not wash too vigorously or the smear itself may come off. Preserve the slides in a cool and dry place in a wooden box to avoid exposure to light and dust. Exposure to light and dust can result in fading of the red colour of the stained TB bacilli.

If there are discrepancies between your reading and that of your supervisor, review these together so that you can learn. Your supervisor will also help you make sure that your staining technique is correct, and is neither too light nor too dark.

After your supervisor has reviewed the slides, the positive slides should be broken and disposed of like other glass scrap. These should never be re-used for any other purpose. Negative slides can either be disposed of, or washed and re-used for non-TB work (e.g. malaria, haematology). You should never re-use TB slides for TB work. If the slide was read wrongly the first time, AFB may be present and may give an incorrect result the second time. In addition, scratches can cause false-positive readings for AFB. For these reasons, only new slides should be used for TB work.

EXERCISE 2

1. How will you explain to a patient to bring up sputum?

   _____

   _____

2. What are the characteristics of a good quality sputum sample?

   (i) _____

   (ii) _____

3. What are the characteristics of a poor quality sputum sample?

   (i) _____

   (ii) _____

4. What may happen if you forget to fix the smear?

   _____

5. What may happen if you fix the smear before waiting for the sputum to dry?

   _____

6. What may happen if you do not filter carbol fuchsin?

   _____

7. What may happen if you forget to heat the slide after adding the carbol fuchsin solution?

   _____

8. How will you preserve the stained slides for quality control?

_____

9. Which is the magnification of the oil immersion lens?
   (a) x4
   (b) x10
   (c) x40
   (d) x100

10. You have been told to make sure that the oil immersion lens never touches the slide. You have also been told never to let the oil applicator touch the slide. The reason for both instructions is the same. What is it?

_____

_____

11. What do the letters AFB stand for and why is this term used to describe the TB bacillus?

_____

_____

12. Please indicate the proper grading of each of the following:

   (i) 20 oil immersion fields with 100 AFB seen.
   Result: _____ Grade: _____

   (ii) 20 oil immersion fields with 30 AFB seen.
   Result: _____ Grade: _____

   (iii) 20 oil immersion fields with 6 AFB seen.
   Result: _____ Grade: _____

   (iv) 20 oil immersion fields with 200 AFB seen.
   Result: _____ Grade: _____

13. List 10 causes of false-positive AFB results:

(i) _____

(ii) _____

(iii) _____

(iv) _____

(v) _____

(vi) _____

(vii) _____

(viii) _____

(ix) _____

(x) _____

14. What may happen as a result of a false-positive AFB result?

_____

15. List 10 causes of false-negative AFB results:

(i) _____

(ii) _____

(iii) _____

(iv) _____

(v) _____

(vi) _____

(vii) _____

(viii) _____

(ix) _____

(x) _____

16. What may happen as a result of a false-negative AFB result?

_____

17. Is the Laboratory Form on the next page correctly filled? If not, what is wrong with it?

18. Using the Laboratory Forms on pages 35–38, complete the first three lines of the Laboratory Register on page 39.

19. There is an error in recording or testing in every line of the Laboratory Register on page 40. Find the errors and indicate the possible implications of each.

Error	Possible implications
1.	
2.	
3.	
4.	
5.	
6.	
7.	
8.	
9.	
10.	

REVISED NATIONAL TUBERCULOSIS CONTROL PROGRAMME

Laboratory Form for Sputum Examination

Name of Health Centre: 237 Date: 3-9-96

Name of patient: Kalavati Age: 16 Sex: M ☐ F ☑

Complete address: 705 Parvati Marg
Gangarh

Patient's TB No.*: _____

Disease classification: ☐ Pulmonary
☐ Extra-pulmonary Site: _____

Reason for examination: ☐ Diagnosis
☑ Follow-up of chemotherapy*

Specimen Identification No.: _____ Date of sputum collection: 4-9-96

Specimen collector's signature Gopal

*Be sure to enter the TB No. for follow-up of patients on chemotherapy.

RESULTS (To be completed in the laboratory)

Lab Serial No: 101

(a) Visual appearance of sputum

	Mucopurulent	Blood-stained	Saliva
Specimen 1	☐	☑	☐
Specimen 2	☑	☐	☐
Specimen 3	☑	☐	☐

(b) Microscopy

Date	Specimen	Results*	Positive (grading)			
			3+	2+	1+	Scanty
3-9-96	1	Pos			✓	
4-9-96	2	Pos		✓		
4-9-96	3	Neg				

* Write negative or positive

Date: 4-9-96 Examined by (signature): Gopal

The completed form (with results) should be sent to the Health Centre to record the results on the Treatment Card.

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REVISED NATIONAL TUBERCULOSIS CONTROL PROGRAMME

Laboratory Form for Sputum Examination

Name of Health Centre: 101 Date: 3-9-96

Name of patient: Lakshmi Kumari Age: 46 Sex: M ☐ F ☑

Complete address: 223, Gandhi Dham
Bapu Nagar

Patient's TB No.*: _____

Disease classification: ☑ Pulmonary
☐ Extra-pulmonary Site: _____

Reason for examination: ☑ Diagnosis
☐ Follow-up of chemotherapy*

Specimen Identification No.: 1 C Date of sputum collection: 4-9-96

Specimen collector's signature Shyam

*Be sure to enter the TB No. for follow-up of patients on chemotherapy.

RESULTS (To be completed in the laboratory)

Lab Serial No: 102

(a) Visual appearance of sputum

	Mucopurulent	Blood-stained	Saliva
Specimen 1	☐	☑	☐
Specimen 2	☑	☐	☐
Specimen 3	☑	☐	☐

(b) Microscopy

Date	Specimen	Results*	Positive (grading)			
			3+	2+	1+	Scanty
3-9-96	1	Pos		✓		
4-9-96	2	Pos		✓		
4-9-96	3	Pos			✓	

* Write negative or positive

Date: 4-9-96 Examined by (signature): Joshi

The completed form (with results) should be sent to the Health Centre to record the results on the Treatment Card.

2/97

REVISED NATIONAL TUBERCULOSIS CONTROL PROGRAMME

Laboratory Form for Sputum Examination

Name of Health Centre: 237 Date: 3-9-96

Name of patient: Lallan Prasad Parmar Age: 51 Sex: M ☑ F ☐

Complete address: 217, Gali Akara
Near Revoli

Patient's TB No.*: _____

Disease classification: ☑ Pulmonary
☐ Extra-pulmonary Site: _____

Reason for examination: ☑ Diagnosis
☐ Follow-up of chemotherapy*

Specimen Identification No.: _____ Date of sputum collection: 4-9-96

Specimen collector's signature Kamala

*Be sure to enter the TB No. for follow-up of patients on chemotherapy.

RESULTS (To be completed in the laboratory)

Lab Serial No: 103

(a) Visual appearance of sputum

	Mucopurulent	Blood-stained	Saliva
Specimen 1	☑	☐	☐
Specimen 2	☑	☐	☐
Specimen 3	☑	☐	☐

(b) Microscopy

Date	Specimen	Results*	Positive (grading)			
			3+	2+	1+	Scanty
3-9-96	1	Pos			✓	
4-9-96	2	Neg				
4-9-96	3	Neg				

* Write negative or positive

Date: 4-9-96 Examined by (signature): Joshi

The completed form (with results) should be sent to the Health Centre to record the results on the Treatment Card.

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REVISED NATIONAL TUBERCULOSIS CONTROL PROGRAMME

Laboratory Form for Sputum Examination

Name of Health Centre: 237 Date: 3-9-96

Name of patient: Srinivasa Rao Age: 36 Sex: M ☑ F ☐

Complete address: WB 2451, Gali Pathanwali
Loni Village

Patient's TB No.*: _____

Disease classification: ☑ Pulmonary
☐ Extra-pulmonary Site: _____

Reason for examination: ☑ Diagnosis
☐ Follow-up of chemotherapy*

Specimen Identification No.: _____ Date of sputum collection: 4-9-96

Specimen collector's signature Kamala

*Be sure to enter the TB No. for follow-up of patients on chemotherapy.

RESULTS (To be completed in the laboratory)

Lab Serial No: 104

(a) Visual appearance of sputum

	Mucopurulent	Blood-stained	Saliva
Specimen 1	☑	☐	☐
Specimen 2	☑	☐	☐
Specimen 3	☑	☐	☐

(b) Microscopy

Date	Specimen	Results*	Positive (grading)			
			3+	2+	1+	Scanty
3-9-96	1	Neg				
4-9-96	2	Neg				
4-9-96	3	Neg				

* Write negative or positive

Date: 4-9-96 Examined by (signature): Joshi

The completed form (with results) should be sent to the Health Centre to record the results on the Treatment Card.

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REVISED NATIONAL TUBERCULOSIS CONTROL PROGRAMME

Laboratory Register

Year _____

Lab Serial No.	Date	Name (in full)	Sex M / F	Age	Complete address (for new patients)	Name of Referring Health Centre	Reason for Examination*		Results			Signature	Remarks
							Diagnosis	Follow-up	1	2	3		

* If sputum is for diagnosis, put a tick (✓) mark in the space under "Diagnosis".
If sputum is for follow-up of patients on treatment, write the patient's TB No. in the space under "Follow-up".

LABORATORY REGISTER WITH ERRORS

REVISED NATIONAL TUBERCULOSIS CONTROL PROGRAMME

Laboratory Register

Year 1997

Lab Serial No.	Date	Name (in full)	Sex M / F	Age	Complete address (for new patients)	Name of Referring Health Centre	Reason for Examination*		Results			Signature	Remarks
							Diagnosis	Follow-up	1	2	3		
499	30/3	Sita Dixit	F		H.No. 211, Pocket III Mayur Vihar	Modern TB Clinic	✓		Neg	Neg	Neg	Joshi	
500	30/3	Krishna Kanth	F	54	H.No. 40, Sector II Jamnagar	Jamnagar Health Centre	✓		Neg	Neg		Joshi	
501	30/3	Aswani Rai	F	39	225, Block 4 Bapu Nagar	Chest Disease Health Centre	✓		3+			Joshi	
502	30/3	Abdul Hazan	M	44	Gali No. 7 J.J. Ram Rani Colony	Aligarh Dispensary		20	Neg			Joshi	
503	1/4	Bhim Singh		38	H.No. 422 Sector-III, Rohini	Modern TB Clinic		102	Neg	Neg		Joshi	
504	1/4	Alex Chopra	M	45		Good Health Centre	✓		1+	1+	2+	Joshi	
505	1/4	Renu Sharma	F	37		Jamnagar Health Centre		✓	1+	2+		Joshi	
506	2/4	Kumar Bhatia	M	58	BB22/Block 4 Nehru Place	Chest Disease Heath Centre	✓		Neg	Neg	Neg		
507	2/4	Deepak Dhawan	M	28		Modern TB Clinic	✓		1+	2+		Joshi	
508	3/4	Preeti Chandra	F	26	H.No. 62, Lane No. 820, Kailash Colony		✓		Neg	Neg	Neg	Joshi	

* If sputum is for diagnosis, put a tick (✓) mark in the space under "Diagnosis".
If sputum is for follow-up of patients on treatment, write the patient's TB No. in the space under "Follow-up".

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20. Next to each step, indicate the reason for the step and whether not performing it correctly could lead to false-positive result, false-negative result, both, or neither and indicate the reason for this.

Steps of the staining procedure	**Reason for the step**	**Consequences if not performed correctly**
Spread sputum on the slide using a broomstick		
Allow the slide to air dry for 15–30 minutes		
Fix the slide by passing it over a flame 3–5 times for 3–4 seconds each time		
Pour filtered carbol fuchsin to cover the entire slide		

Steps of the staining procedure	Reason for the step	Consequences if not performed correctly
Gently heat the slide with carbol fuchsin on it until vapours rise. DO NOT BOIL		
Leave the slide for 5 minutes		
Rinse GENTLY with tap water until all free carbol fuchsin stain is washed away		
Tilt the slide to drain off excess water		
Pour 25% sulphuric acid onto the slide		
Let the slide stand for 2–4 minutes		

Steps of the staining procedure	Reason for the step	Consequences if not performed correctly
Rinse GENTLY with tap water until all free stain is washed away		
Pour 0.1% methylene blue onto the slide		
Let the slide stand for 30 seconds		
Rinse GENTLY with tap water		
Allow the slide to dry and then examine it under the microscope		

HOW TO DISPOSE OF CONTAMINATED MATERIALS SAFELY

Sputum specimens examined in the laboratory are potentially infectious and after examination these must be disinfected and destroyed so that risk of infection is avoided. **All disposable containers are used only once. Positive slides should never be used again and should be destroyed.**

After the smears are examined, remove the lids from all sputum cups and put the cups and removed lids in a bucket containing 5% hypochlorite or 5% phenol solution. The cups and lids should be fully submerged in the solution. Similarly, used broomsticks should also be put in the same bucket containing 5% hypochlorite or 5% phenol solution. The bin/bucket should have a foot-operated lid. Thereafter, the used sputum cups, lids and wooden sticks can be disposed of by any of the following methods:

1. Autoclaving in an autoclave or in a pressure cooker. At the end of the laboratory work the sputum cups and the removed lids, along with broomsticks can be placed in a pressure cooker of approximately 7 litre capacity containing adequate amount of water to submerge the contents and boiled for at least 20 minutes using any heating source, electrical or non-electrical. After proper cooling the material can be discarded with other waste.
2. If autoclaving cannot be done, use chemicals such as freshly prepared 5% hypochlorite solution or 5% phenol. Caps of the sputum cups must be removed and the cups, caps and broomsticks submerged in the solution in a secure place overnight. After this, the solution, cups, caps and broomsticks can be discarded with other waste.
3. As a last resort, if none of the above is available, sputum cups, caps and broomsticks can be burnt in a pit at a safe distance away from inhabited areas.

HEALTH EDUCATION AND COMMUNICATION WITH PATIENTS

DOs	DON'Ts
• Communicate respectfully and patiently with patients • Explain and demonstrate to patients by actions the method of bringing out sputum • Examine the quality of sputum samples before patients leave the laboratory • Tell patients that TB is curable if regular and complete treatment is taken • Tell patients that treatment for TB is free of cost • Tell patients to tell others with symptoms of TB to contact the health facility	• Tell patients that their test for TB is negative • Tell patients that they are cured • Be impatient or rude with patients • Refuse to accept sputum from patients at any time of the day • Give the results of sputum examination only to the patient • Make tuberculosis patients feel rejected

Patients with tuberculosis may be highly infectious, but if they take effective treatment using DOTS they themselves will be cured and will also not infect others. The LT must be aware that patients with negative sputum smears may have tuberculosis. Therefore, they should never tell patients that their test for tuberculosis is negative. Patients with negative sputum smears must be further examined by a Medical Officer. The Medical Officer will determine if they have tuberculosis or not. Similarly, when follow-up sputum is examined, the LT must never tell

patients that their tuberculosis is cured. Although smears become negative within 2–3 months in most patients on DOTS, they will not be cured unless they complete a full course of treatment. Patients who stop treatment before they have completed a full course of treatment are likely to develop even more severe disease.

Collection of sputum in the correct manner is essential both for diagnosing and monitoring treatment of tuberculosis. By communicating effectively with patients, the LT improves patients' diagnosis and treatment. By showing respect and patience when talking with patients, the LT can encourage patients to take treatment until they are cured.

Annexure I

REVISED NATIONAL TUBERCULOSIS CONTROL PROGRAMME

Laboratory Form for Sputum Examination

Name of Health Centre: _____ Date: _____

Name of patient: _____ Age: _____ Sex: M ❑ F ❑

Complete address: _____

_____

Patient's TB No.*: _____

Disease classification: ❑ Pulmonary

❑ Extra-pulmonary Site: _____

Reason for examination: ❑ Diagnosis

❑ Follow-up of chemotherapy*

Specimen Identification No.: _____ Date of sputum collection: _____

Specimen collector's signature _____

*Be sure to enter the TB No. for follow-up of patients on chemotherapy.

RESULTS (To be completed in the laboratory)

Lab Serial No: _____

(a) Visual appearance of sputum

	Mucopurulent	Blood-stained	Saliva
Specimen 1	❑	❑	❑
Specimen 2	❑	❑	❑
Specimen 3	❑	❑	❑

(b) Microscopy

Date	Specimen	Results*	Positive (grading)			
			3+	2+	1+	Scanty
	1					
	2					
	3					

* Write negative or positive

Date: _____ Examined by (signature): _____

The completed form (with results) should be sent to the Health Centre to record the results on the Treatment Card.

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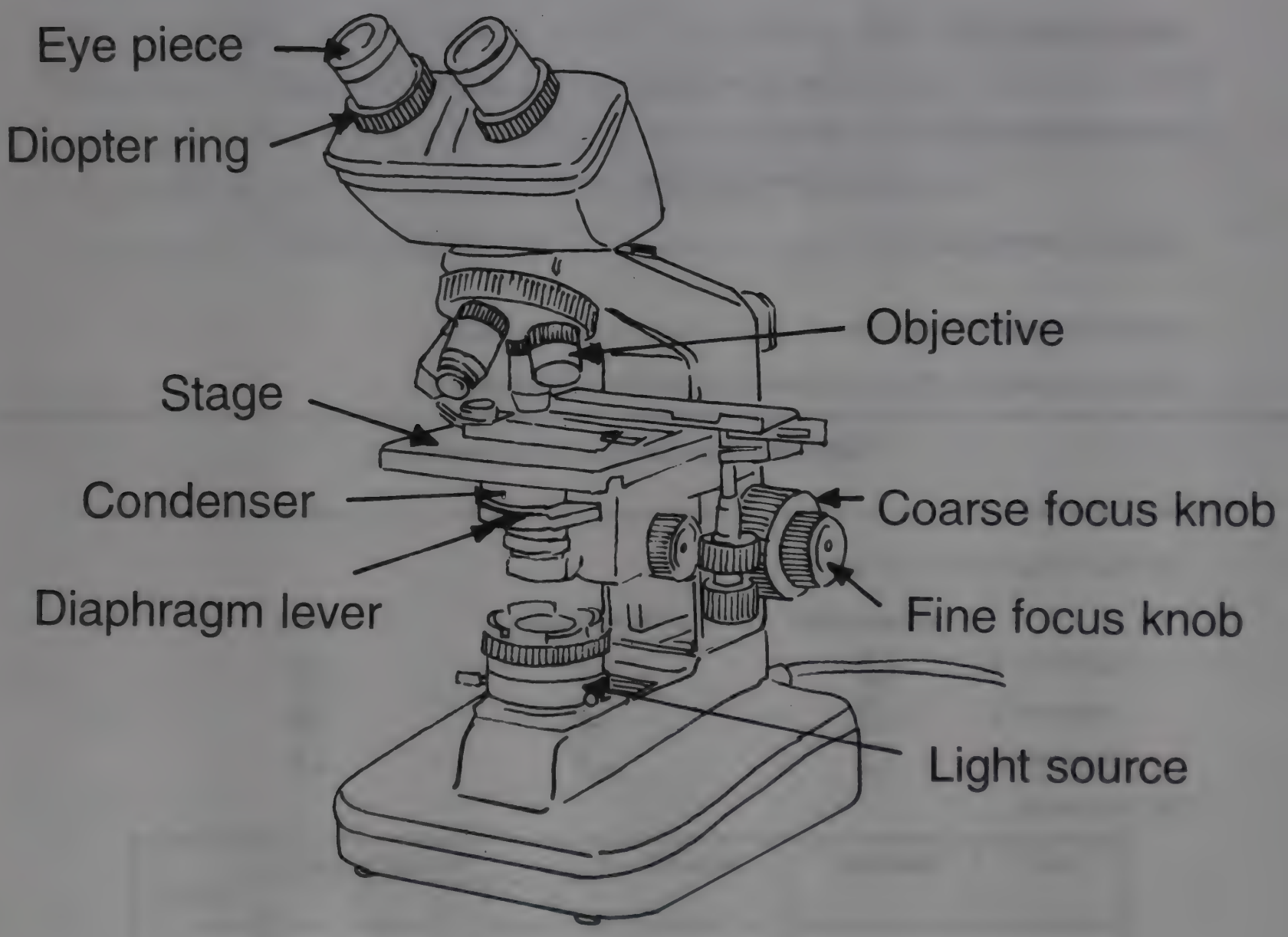
Annexure II

Microscope and its parts



Annexure III

Care of the Microscope

The microscope is the lifeline of the Revised National Tuberculosis Control Programme. Proper handling and maintenance of the microscope, particularly of its lenses, is very important. The following points should be observed:

1. Place and store the microscope in a dry, dust-free and vibration-free environment.

- Vibration damages the microscope.
- When the microscope is not being used, cover or keep it in the box so as to keep it free from dust.
- Avoid exposing the microscope to direct sunlight.
- Avoid exposing the microscope to moisture. Humidity may allow fungus to grow on the lens and cause rusting of the metal parts.
- Put plenty of dry blue silica gel into a shallow plate and place it in the box when the microscope is kept in it. Silica gel is blue in colour when it is dry but when it becomes wet it turns pinkish. As soon as the silica gel becomes pink, change or heat it until it turns blue again and then reuse it.

2. Keep the microscope and lenses clean.

- Clean the microscope with lens paper before and after use.
- Do not leave immersion oil on the surface of the immersion lens.
- Never use spirit or alcohol to clean the lenses, as these can damage them.
- Never let the oil immersion lens touch the smear.
- Use the fine focusing knob only while using the oil immersion lens.
- All the lenses should be cleaned with dry lens paper. Lens paper can be moistened with xylene if necessary. Do not clean lenses with an ordinary cloth.

Annexure IV

Items Needed for Staining and Examining Slides for AFB

For preparing the sputum smear:

- Wooden broomsticks for spreading sputum
- Carbol fuchsin solution in a plastic squeeze bottle (500 ml capacity)
- 25% sulphuric acid (H_2S0_4) in a plastic squeeze bottle (500 ml capacity)
- 0.1% methylene blue solution in a plastic squeeze bottle (500 ml capacity)
- Staining rack
- Heat source (spirit lamp or gas burner)
- Tap water

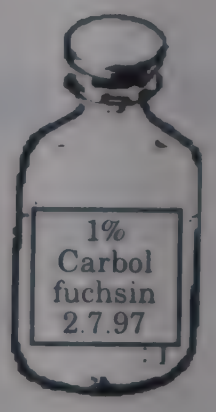
For microscopic examination:

- Diamond marker or glass marking pencil to label slides
- Immersion oil for x100 examination
- Xylene to clean slides and microscope lens
- Lens paper to clean the microscope lens

Annexure V

Formulation of Reagents

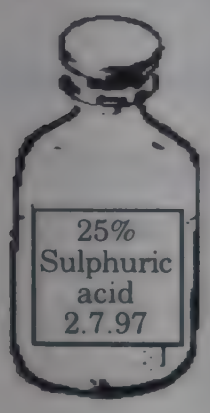
Preparation of 1% carbol fuchsin



- Weigh 5 grams of basic fuchsin dye in a balance and transfer it to 250 ml Ehrlenmeyer glass flask.
- Add 50 ml of methylated spirit and shake to dissolve the dye.
- Heat 25 grams of phenol to melt it and add it to the above solution.
- Heat the flask containing basic fuchsin dye dissolved in spirit and phenol gently in a water bath at about 60°C. **Do not heat directly on a flame**.
- Transfer the contents into a 500 ml measuring cylinder.
- Add distilled water to make up a final volume of 500 ml.
- Pour the solution through filter paper (Whatmann No. 1) and store filtered solution in a glass bottle. Label the bottle as 1% carbol fuchsin with the date of preparation.

Any time particles start to form in carbol fuchsin solution, the solution must be filtered again.

Preparation of 25% sulphuric acid



- Pour 375 ml of distilled water into a 1 litre glass flask.
- Measure 125 ml of concentrated sulphuric acid and transfer it slowly into the flask containing water.
- Always add acid to water. Never add water to acid.
- Store the sulphuric acid solution in a labelled glass bottle.

Preparation of 0.1% methylene blue solution

- Weigh 0.5 grams of methylene blue and transfer to a 1 litre glass flask.
- Add 500 ml of distilled water.
- Shake well to dissolve.
- Store in a glass bottle with the label showing name of the reagent and date of preparation.

Annexure VI

Prevention and Consequences of False-positive and False-negative Sputum Results

HOW TO PREVENT FALSE-POSITIVE SPUTUM RESULTS

- Always use new, unscratched slides
- Use a separate broomstick for each sample
- Always use filtered carbol fuchsin
- Do not allow the carbol fuchsin to dry during staining
- Decolourize adequately with sulphuric acid
- Make sure there are no food particles or fibres in the sputum sample
- Never allow the oil immersion applicator to touch a slide
- Never allow the oil immersion lens to touch a slide
- Label sputum containers, slides and Laboratory Forms accurately
- Cross-check the number on the Laboratory Form and sputum container before recording
- Record and report results accurately

Consequences of false-positive sputum results

- Patients are begun on treatment unnecessarily
- Treatment is continued longer than necessary, in the case of follow-up examinations
- Medications will be wasted
- Patients may lose confidence in the Programme

HOW TO PREVENT FALSE-NEGATIVE SPUTUM RESULTS

- Make sure the sample contains sputum, not just saliva
- Make sure there is enough sputum (at least 2 ml)
- Select thick, purulent particles to make the smear
- Prepare smears correctly—not too thick, too thin or too little material
- Fix the slide for the correct length of time, not too short or too long
- Stain with carbol fuchsin for the full 5 minutes
- Do not decolourize with sulphuric acid too intensively
- Examine every smear for at least five minutes before recording it as negative
- Label the sputum containers, slides and Laboratory Forms carefully
- Cross check the number on the Laboratory Form and sputum container before recording
- Record and report results accurately

Consequences of false-negative sputum results

- Patients with TB may not be treated, resulting in suffering, spread of TB and death
- Intensive phase treatment may not be extended for the required duration, resulting in inadequate treatment
- Patients may lose confidence in the Programme

Annexure VII

Job Responsibilities of the Laboratory Technician (LT) in the Revised National Tuberculosis Control Programme

1. Sputum collection
- Demonstrate to patients how to bring out good quality sputum.
- Label the sputum container properly.
- Before the patient leaves, check the sample to see if it is sputum or only saliva.

2. Sputum processing and examination
- Write the Laboratory No. and visual appearance of the sputum on the Laboratory Form.
- Always use new slides.
- Spread the smear and heat it in order to fix it on the slide.
- Stain the smear by the Ziehl-Neelsen method.
- Examine the stained smear under the microscope.

3. Recording and reporting
- Enter the result of each microscopic examination on the Laboratory Form and in the Laboratory Register.
- Maintain the Laboratory Register properly, including the reason for sputum examination.
- Send the Laboratory Form with results recorded to the treating physician promptly.

4. Quality control
- Preserve all positive and negative slides until they are reviewed by the Supervisor.

5. Safety
- Keep the laboratory clean.
- Do not eat, drink or smoke in the laboratory.
- Safely dispose of all contaminated materials including sputum cups.
- Break all positive slides after they have been cross-checked by the supervisor.

6. Material management
- Keep the microscope in good working condition.
- Prepare and store solutions and reagents properly.
- Order supplies well in advance to avoid shortages.
- Use freshly prepared reagents

Wash hands every time you handle contaminated material

Annexure VIII

Ziehl–Neelsen Staining

1. Select a new unscratched slide and label the slide with the Laboratory Serial Number.
2. Spread sputum on the slide using a broomstick.
3. Allow the slide to air dry for 15–30 minutes.
4. Fix the slide by passing it over a flame 3–5 times for 3–4 seconds each time.
5. Pour filtered carbol fuchsin to cover the entire slide.
6. Gently heat the slide with carbol fuchsin on it until vapours rise. Do not boil.
7. Leave carbol fuchsin on the slide for 5 minutes.
8. Gently rinse the slide with tap water until all free carbol fuchsin stain is washed away.
9. Pour 25% sulphuric acid onto the slide.
10. Let the slide stand for 2–4 minutes.
11. Rinse gently with tap water. Tilt the slide to drain off the water.
12. If the slide is still red, reapply sulphuric acid for 1–3 minutes and rinse gently with tap water.
13. Pour 0.1% methylene blue onto the slide.
14. Leave methylene blue on the slide for 30 seconds.
15. Rinse gently with tap water.
16. Allow the slide to dry.
17. Examine the slide under the microscope using x40 lens to select the suitable area and then examine under x100 lens using a drop of immersion oil.
18. Record the results in the Laboratory Form and the Laboratory Register appropriately as per the table given below:

Examination	Result	Grading	No. of fields to be examined
More than 10 AFB per oil immersion field	Pos	3 +	20
1–10 AFB per oil immersion field	Pos	2 +	50
10–99 AFB per 100 oil immersion fields	Pos	1 +	100
1–9 AFB per 100 oil immersion fields	Scanty	Record exact number seen	200
No AFB in 100 oil immersion fields	Neg	0	100

19. Store all positive and negative slides until instructed by the supervisor.
20. Disinfect all contaminated materials before discarding.
